REMARKS

Claims 72, 79, and 85 have been canceled. Claims 68 and 101-103 have been amended. The application now includes claims 66-68, 70, 71, 73-78, 80-84, and 86-107.

In order to simplify prosecution, claims 72, 79, and 85 have been canceled. This makes moot the rejection of these claims under 35 U.S.C. 112, first paragraph. The applicant retains the right to pursue these claims, or similar claims, in one or more continuing applications. As acknowledged in the office action, there is at least one example taught in the application of a pharmaceutical active being more soluble in the cubic phase when the essential oil is present. Moreover, in the patent application, in item 4 of the declaration of Dr. Anderson filed September 15, 2010, and in Exhibit 5 of the declaration of Dr. Anderson filed March 26, 2009, it is demonstrated that the inclusion of an essential oil or tocopherol is necessary for the formation of reverse cubic phases in the case where the lipid is phosphatidylcholine and for high levels of loading. That is, without the presence of an essential oil or tocopherol, the compositions of water, phospholipid, and a difficult to solubilize pharmaceutical do not form reverse cubic phase materials, nor do they contain high loadings of difficult to solubilize pharmaceuticals in the reverse cubic phase structured fluid.

In order to simplify prosecution, claims 68 and 101-103 have been amended to eliminate the reference "excluding anisole". As discussed previously with the Examiner during the Examiner's Interview prior to filing the amendment, "anisole" is not an essential oil. Therefore, the inclusion of this requirement, which was discussed during the interview, was redundant. This amendment should therefore obviate the rejection of claims 68, 83-89, 92, 94, 97 and 101-103 under 35 U.S.C. 112, first paragraph.

It should be clear from this record that claims 68 and 101-103 do not cover anisole.

Paragraph [0211] and [0212] specifically defines "essential oils" as including allspice berry, amber essence, anise seed, arnica, balsam of peru, basil, bay, bay leaf, bergamot, bois de rose (rosewood), cajeput, calendula (marigold pot), white camphor, caraway seed, cardamon, carrot seed, cedarwood, celery, german or hungarian chamomile, roman or english chamomile, cinnamon, citronella, clary sage, clovebud, coriander, cumin, cypress, eucalyptus, fennel, siberian fir needle, frankincense (olibanum oil), garlic, rose geranium, ginger, grapefruit, hyssop, jasmine, jojoba, juniper berry, lavender, lemon, lemongrass, lime, marjoram, mugwort, mullein flower, myrrh gum, bigarade neroli, nutmeg, bitter orange, sweet orange, oregano

palmarosa, patchouly, pennyroyal, black pepper, peppermint, petite grain, pine needle, poke root, rose absolute, rosehip seed, rosemary, sage, dalmation sage, santalwood oil, sassafras, spearmint, spikenard, spruce (hemlock), tangerine, tea tree, thuja (cedar leaf), thyme, vanilla extract, vetivert, wintergreen, witch hazel (hamamelia) extract, ylang ylang (cananga) extract and components and mixtures thereof. (emphasis added)

It should be noted that this definition includes "anise seed", not "anisole". Pages 106 and 1072 of the Merck Index, attached to September 15, 2010, declaration of Anderson explicitly show the oil of anise, which comes from anise seed, is chemically distinct from anisole. Moreover, these pages demonstrate anisole is a synthetic. Thus, the Examiners reliance on MPEP 2112.02 "Products of identical chemical composition can not have mutually exclusive properties" does not apply to the present record which clearly establishes "anisole" is not anise seed (or oil of anise). As noted in point 9 of the declaration of Anderson filed September 15, 2010, anisole is obtained by synthesis not from a plant source. The CFR also identifies anisole as a synthetic flavoring agent. Hence, it is simply improper for the Examiner to take the position that anisole reads on a component of an essential oil.

The references relied on by the Examiner, Muldoon and Burdock, in fact, teach exactly the opposite conclusion reached by the Examiner. Specifically, Muldoon teaches the reaction of sodium phenoxide with methylsulfate to produce anisole, and Burdock teaches a number of different materials, NONE of which contain any essential oils (see declaration of Anderson at point 9C). The Examiner has cited Muldoon as definitive in the issue of anisole in natural plant sources. However, Muldoon simply references H. Maarse's book Volatile Compounds in Foods and Beverages [(1991) Marcel Dekker, New York, NY], which contains discussions of anisole-derivatives, not anisole. Page 715 of Maarse (attached to this response) contains the following, which demonstrates the manner in which anisoles are referred to in Maarse:

"This bleaching is done with chlorine, which reacts with phenol to give 2,4,6-trichlorophenol. The phenol is transformed by molds into its anisole. However, other sources may also cause the presence of chloroanisoles …".

It should be noted that this passage does not in any way refer to anisole itself, merely compounds that contain the anisole motif within the (larger) molecule. Thus, when the OH in a phenolic compound is changed to OCH_3, this creates an anisolic compound, or 'an anisole' for short—not anisole itself. This is true of other discussions in Maarse, where anisoles, not anisole, are mentioned. Digging further, in his discussion of artichoke volatile components, Maarse

references Buttery et al. [(1978) J. Agric. Food. Chem. 26(4):791] (copy attached for the convenience of the Examiner), which lists the volatile components (i.e., essential oil components) of artichoke, but nowhere does anisole (also called methoxybenzene) appear. Indeed, Table I of that publication, which lists the compounds detected by capillary GLC and is entitled "Identities of Constituents of the Steam Volatile Oil of Artichokes", does not show anisole, nor methoxybenzene, nor anisole under any other name. Thus, it appears that the Examiner has mistakenly deduced that any oil containing anisoles must contain anisole itself, which is simply not true.

The applicant has performed a careful review of the entries on Globe Artichoke (page 246) and Jerusalem Artichoke (page 223, and 231) (copies attached) in Maarse and found no mention of anisole. Pages 715 and 707 appear to be the only places where the root -anisole- appears in Maarse, and in both cases the reference is to anisoles, not anisole itself.

Claims 66, 70, 71, 73, 74, 90, 95, 98, 100, 104, and 106 were rejected as being anticipated by Anderson WO 9912640 as evidenced by U.S. Patent 5,026,548 to Evans, Burdock, and Muldoon. Claims 67, 68, 72, 75-89, 91, 93, 94, 97, 97, 99, 101-103 were rejected as being obvious over Anderson, as evidenced by Evans, Burdock, and Muldoon. These rejections are traversed.

Rather, than support a case for anticipation or obviousness, Anderson in fact teaches the unobviousness of the claimed invention. All claims in the instant invention REQUIRE a reversed cubic phase. On page 7 of the office action, the Examiner points to Example 36 of Anderson and notes that Anderson explicitly states in the examples that if the concentration of paclitaxel in this system were lowered then the solubilization of paclitaxel becomes a truly stable solubilization. Of course, this completely overlooks the fact that in Example 36 there is taught a nanostructured liquid phase, not a reversed cubic phase. This is stated in the first sentence of Example 36. As discussed in the response and declaration of Dr. Anderson filed December 15, 2009, additional confirmatory experiments were conducted which demonstrated a liquid phase was formed not a reversed cubic phase, and it was noted that a much higher loading was achieved when essential oils were used and reversed cubic phases were formed.

Indeed, the formation of a liquid phase—in other words, *liquefaction*—upon the addition of a powerful solubilizer of the type taught in the instant invention is the norm in the field of surfactant and lipid phase behavior, and circumventing this so as to allow high loadings of

powerful solubilizers in reversed cubic *liquid crystalline* materials and their unique advantages lies at the very heart of the instant invention. When an essential oil or other powerful solubilizer is incorporated into a lipid-water or surfactant-water system at meaningful levels, there is a strong tendency for the phase to liquefy. After all, in the case of a difficult-to-solubilize drug like paclitaxel, *if the solubilizing power of an essential oil is strong enough to dissolve the drug, chances are it is strong enough to liquefy—dissolve—the lipid phase.* The prior art paradigm is that co-solubilizers (referred to as co-surfactants, oils, or "third components") induce the formation of liquid phases such as microemulsions and micellar solutions as their loading increases.

Evans in fact illustrates this prior art paradigm, since, in the presence of the surfactants being extracted (and of course water), the alcohols used in the Evans process induce liquefaction of the surfactant-rich phase, and this is in fact an important convenience of the process. In the 1940's and 50's, cubic phases were thought of as nuisances in the soap and surfactants industry because of their high viscosities, and the genesis of their study was largely motivated by the desire to understand them so as to be able to eliminate their presence from the industrial processes utilized. So, in fact, the weight of the prior art teaches that "third components" should actually be chosen so as to liquefy the surfactant-rich materials and *avoid* high-viscosity phases such as cubic phases. In short, Evans, along with much of the prior art, teaches away from the instant invention in promoting liquefaction and avoiding cubic phases.

The Anderson prior art, insofar as it makes use of third components, neither avoids nor promotes liquid crystalline phases over liquid phases, but rather treats them as all being amenable to the coatings which are the focus in that disclosure. And in particular, as mentioned above, the Example 36 specifically cited by the Examiner is a case in which the third component induces liquefaction, i.e., the formation of a liquid phase. Paclitaxel is notoriously difficult to solubilize and to retain in solution, as it has a strong tendency to precipitate out of solution over timeframes of hours or days (that is, the solution is metastable). Thus it is not surprising that in the Examples of Anderson, when significant loadings of paclitaxel are attempted, either the paclitaxel is found to precipitate out over time, or, if the essential oil is at high enough loading to enable true, stable solubilization of this active, then the overall surfactant-rich phase liquefies.

In the pharmaceutical world, particularly in injectable products, it should be particularly noted that the two most commonly used lipids in connection with cubic phases, namely glycerol

monooleate (GMO, and related monoglycerides) and phosphatidylcholine, are not recognized to form cubic phases when combined with trigylcerides (fats), which are far and away the most commonly used oils in pharmaceuticals, especially injectables. The addition of anything more than 2% triglyceride to the well-known GMO/water cubic phase—which is the object of study in well over 50% of the publications on cubic phases—will transform the cubic phase to a different phase. And the fact that phosphatidylcholine / water systems form lamellar phases and associated emulsions upon addition of triglycerides is almost legendary, and is the basis of many injectable formulations, such as Intralipid for parenteral nutrition and Propofol for Injection (e.g., Diprivan). In sharp contrast, the present invention shows, describes and claims reversed cubic phases which employ essential oils or tocopherol.

A survey of surfactant-water phase diagrams from the prior art will reveal that by and large, the cubic phases are the most prone of all the liquid crystalline phases to liquefaction, whether by a third component, impurities in the surfactant, temperature, or by other changes in composition. Cubic phase regions in phase diagrams have long been known to be very small in extent (i.e., tolerate only small changes in composition). Part of the explanation for this lies in the fact that, over a lattice fundamental unit of the cubic phase structure, the local properties (molecular packing and interactions, as quantified by the principal curvatures) vary from point to point, in contrast with the higher-symmetry hexagonal and particularly the lamellar phase, making the cubic phase entropically less favorable (not to mention so-called "frustration" effects, also disfavoring the cubic phase). What is remarkable, and surprising, about the instant invention is that it teaches a broad range of reversed cubic liquid crystalline phases which are thermodynamically stable and contain high loadings of pharmaceutically acceptable co-solubilizer and solubilized drug, in spite of prior art teachings that "oily" third components are to be associated with liquids such as microemulsions and micellar solutions.

In short, contrary to the position on page 7 of the office action, Anderson does <u>not</u> exemplify anywhere a composition comprising the same claimed components—Example 36 of Anderson does not have the claimed reversed cubic phase. Rather, Example 36 would stand for the proposition that reversed cubic liquid crystalline phases are <u>not</u> made by the procedures in Example 36.

Example 37 was also erroneously analyzed in the office action. As noted in point 6 in the declaration of Dr. Anderson filed December 15, 2009, Example 37 shows the formation of a

metastable phase. As explained in the declaration of Dr. Anderson filed December 15, 2009, this means that the drug rapidly precipitates into the surrounding liquid phase leaving very low loading of the drug in a stable reversed cubic phase (a problem specifically overcome by the present invention). As noted above, anisole is not an essential oil; however, even if it were erroneously assumed to be, Example 37 still does not teach the claimed invention which requires the reversed cubic phase to be made from water, phospholipid, essential oil or a component thereof or tocopherol, and a difficult to solubilize pharmaceutical, as Example 37's own teachings show the "metastable" character which results in the drug going to a liquid phase.

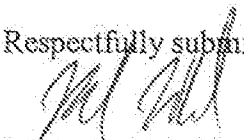
Furthermore, while the figures point 4 of the Anderson declaration filed September 15, 2010 may be difficult to view, there is no mistaking the testimony in point 4 which shows that in metastable materials, the drug precipitates out of solution. These figures are quite clear electronically, and, if desired can be provided by e-mail or in color filed at the USPTO receiving office window if further evaluation is needed.

There is simply no teaching in Anderson that one of ordinary skill in the art could make particles with a reverse cubic phase formed from water, phospholipids, essential oils or tocopherol, and pharmaceutical actives where the pharmaceutical actives are present at a higher loading than if the essential oils or tocopherol was not present.

In view of the above and the concurrently filed declaration, claims 66-68, 70, 71, 73-78, 80-84, and 86-107 should now be reconsidered and allowed at an early dated.

Should the Examiner find the application to be other than in condition for allowance, the Examiner is requested to contact the undersigned at 703-787-9400 (fax: 703-787-7557; e-mail: mike@wcc-ip.com) to discuss any other changes deemed necessary in a telephonic or personal interview.

Respectfully submitted,



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D. Strawberry Flavor

The breeding of fungus-resistant grapevine varieties by hybridizing American wild vines is of great importance. However, the crossing of these wild vines with European cultured vines has often given distasteful off-flavors described as foxy, grassy, medicinal, and so on. By extensive breeding programs, most of these off-flavors could be eliminated. The cause of one of these off-flavors, described as strawberry-like, was investigated by Rapp et al. [190]. They were able to identify two compounds responsible for this flavor: 2,5-dimethyl-4-hydroxy-3(2H)-furanone and 2,5-dimethyl-4-methoxy-3(2H)-furanone. With this result, seedlings forming these undesirable compounds can be identified at an early stage and be excluded from the breeding program.

E. Peppery Flavor

Dubois et al. [191] analyzed freshly distilled rums with a pepper-like aroma. They identified two addition products of ethanol and 2-propenal: 3-ethoxypropanal and 1,1,3-triethoxypropane. These compounds had been found before in peppery whiskeys [192]. This off-flavor also originates from the formation of 2-propenal during fermentation.

F. Oxidized Flavor

Grape marc, or pomace, is the mass of skins, stalks, and seeds left after the winemaking process. This material contains residual sugar, which can be fermented to ethanol. This alcohol, used as fortifying spirit, may have an undesirable flavor. Williams and Strauss [193] found that this was due to the autoxidation of trace amounts of unsaturated fatty acids (from grapeseed oil liberated during pressing operations) to give a series of aldehydes. The spirit could be deodorized by passing it through a strongly basic anion-exchange resin.

G. Migration from Cork

Cork taint is one of the most frequently occurring off-flavors in wine and other beverages. The flavor can be described as musty. It is one of the examples of contamination with chloroanisoles (see also Sec. II.C). In most cases the chloroanisoles are derived from the cork. The isomer found in that case is 2,4,6-trichloroanisole. The explanation for its presence is found in the bleaching of cork to remove colorings and to decrease the total microbial count. This bleaching is done with chlorine, which reacts with phenol to give 2,4,6-trichlorophenol. The phenol is transformed by molds into its anisole. However, other sources may also cause the presence of chloroanisoles in alcoholic beverages; these are barrels or pallets from wood treated with chlorophenols.

Simpson et al. [194] report a contamination of cork with guaiacol that had probably taken place during transport or storage. Wine bottled with these corks had obtained a strong phenolic, medicinal taste. Concentrations of guaiacol found in wine ranged from 0.07 to 2.63 mg/liter and in the associated corks from 0.13 to 2.14 mg/cork. The flavor threshold value for guaiacol in dry white wine was found to be 0.020 mg/liter.

Microbial breakdown of paraffin on the corks may lead to petroleum-like odors [195].

Quite a different cause of an oily odor in fish can be the presence of dimethyl sulfide [139]. A characteristic of this type of oily smell is that it occurs only in heated seafood. It is proposed that the precursor is dimethyl-β-propiocetin (DMPT), a compound known to be present in seaweeds. When heated to 90–100°C in faintly alkaline medium, DMPT readily decomposes to form dimethyl sulfide.

Whitfield [140] reports on another source of aromatic hydrocarbons in fish. Fish stored in cold storage rooms that had recently been painted with marine varnish were heavily contaminated with the aromatic hydrocarbons used as solvent.

Besides the fact that petroleum compounds cause off-flavors, some petroleum components are also harmful to animals and plants [135]. Benzene, toluene, and xylenes can inhibit reactions in the energy transfer system, and biochemical membranes can be broken down. Seaweeds can start to abnormally metabolize sulfur compounds. DMPT, which gives a desirable flavor, is decreased to a very low level by petroleum oil contamination.

D. Halogen Compounds

The most common off-flavor due to halogen compounds can be described as medicinal and is caused by chlorophenols (see also Sec. II.C). In the case of drinking water, these compounds are easily formed by the chlorination of water containing phenol [141]. Subsequently this water can contaminate other products when it is used for processing.

Watanabe et al. [142] determined polybrominated anisoles and organochlorine compounds in fish, shellfish, and sediments in Japan. 2,4,6-Tribromoanisole was found in several samples with a maximum level of 5.4 ppb. Its presence could be explained in two different ways, chlorination of water in the presence of bromine and its use as flame retardant, both giving tribromophenol, which is transformed in the corresponding anisole by microorganisms.

Bemelmans and den Braber [143] found that marinated herring was contaminated with o-bromophenol to give an iodine-like and phenolic odor and taste. It was assumed that the herring was already contaminated before harvest. An explanation given by the authors for the presence of this compound was that bromophenols are accumulated or produced by algae.

Whitfield et al. [144] investigated an iodine-like flavor in prawn. They identified 2- and 4-bromophenol, 2,4- and 2,6-dibromophenol, and 2,4,6-tribromophenol in all samples analyzed. The 2,6-dibromophenol was found to possess the typical odor of the off-flavor and to have the lowest flavor threshold value (0.5 ng/liter in water; 60 ng/kg in prawn meat). The same five bromophenol isomers were found in a mixture of marine algae and bryozoa, which indicates them as the most probable source.

E. Microbial Metabolites in Crustaceans

Crustaceans include rock and sand lobsters, crabs, and prawns. Whitfield and Freeman [145] reviewed off-flavors found in these species. Five main types of off-flavors were encountered: kerosene- or solvent-like and iodoform-like flavors, which have been described in the preceding sections, and garlic-like, garlic-metallic, and onion-like flavors. Garlic flavor was caused by bis(methylthio)methane, metallic taste by 1-octen-3-ol and (Z)-1,5-octadien-3-ol, and onion flavor by dimethyl trisulfide. The sulfur com-

With the exception of one compound, benzothiazole, all of the compounds found in broccoli have also been identified in cooked cauliflower. These vegetables belong to the same family; therefore it is likely that the precursors for most of the compounds found in broccoli are the same as those present in cauliflower. The principal difference in the volatile components of these vegetables is in the composition of the isothiocyanates and nitriles. Only two isothiocyanates, the 4-(methylthio)butyl and 2-phenylethyl derivatives, have been identified in broccoli, whereas five, including these two, have been found in cauliflower. Similarly, only two nitriles, those corresponding to the two isothiocyanates, have been found in broccoli compared with four identified in cauliflower. However, seven glucosinolates, including the 3-propenyl derivative, sinigrin, have been identified in broccoli [55]. But no 2-propenyl isothiocyanate or 3-butenenitrile was detected. Clearly, the volatile components of cooked broccoli require further investigation as does the composition of the glucosinolates present in this vegetable. Studies of the volatile components of shredded raw broccoli could also provide some useful information, as would a study of the reaction of cabbage or cauliflower myrosinase on broccoli glucosinolates.

D. Globe Artichoke

The young flower heads of the globe artichoke (Cynaras scolymus L.) are usually eaten hot with the main meal, but in some countries baby artichokes are also eaten raw as an appetizer or preserved in olive oil. Only the volatile components of the cooked vegetable have been studied. The extract was obtained by combined steam distillation/solvent extraction at atmospheric pressure [87,123]. Combined GC-MS was the principal method of identification. These identifications were supported either by relative concentrations of the major components [123] or by quantitative data [87]. Aroma descriptions were obtained in one study [87], but no aromagrams have been published.

A total of about 50 compounds have been identified, of which the major classes of compounds are aliphatic alcohol and carbonyl compounds (19) and mono- and sesquiterpenoids (12). Based on a yield of artichoke oil of 17 μL/kg [123], the major components in these extracts were β-selinene (7 μg/kg), caryophyllene (3 μg/kg), benzyl alcohol (5.8 μg/kg), phenylacetaldehyde (1.2–2.8 μg/kg), α-cedrene (0.7 μg/kg), (Z)-3-hexenol (<0.1–0.6 μg/kg), hexanol (0.1–0.5 μg/kg), eugenol (0.2–0.8 μg/kg), and 3-methyl-2-butenol (0.4 μg/kg). Compounds considered to be most important in the cooked artichoke aroma are 1-octen-3-one (T 5 ng/liter), 1-hexen-3-one (T 20 ng/liter), (E)-2-nonenal (T 80 ng/liter), decanal (T 100 ng/liter), eugenol (T 6 μg/liter) and phenylacetaldehyde (T 4 μg/liter) [123]. Only one of these compounds, 1-hexen-3-one, was described as possessing an aroma characteristic of cooked artichokes. Other sensory studies [87] have, however, suggested that several sesquiterpene hydrocarbons, α-cedrene, ε-muurolene, β-selinene, and α-humulene, play a major role in the aroma of this vegetable. α-Cedrene, because of its relatively high concentration (0.7 μg/kg), is considered the most important [87].

The aliphatic compounds identified in the extracts are typical of most cooked green vegetables and as such are probably derived from the Strecker degradation of amino acids and the oxidative decomposition of unsaturated fatty acids. The sesquiterpene hydrocarbons are, however, most interesting metabolites of this plant, and therefore their biosynthesis possibly warrants investigation.

these compounds in the French fries, for although it was indicated that the oxazole fraction accounted for 0.01% of the headspace extract, the weight of the total extract was not given.

The major alkyloxazoles that were identified were 2,4,5-trimethyloxazole, 2-isopropyl-4,5-dimethyloxazole, 2-methyl-4-ethyl-5-propyloxazole, and 2-ethyl-4-methyl-5-propyloxazole. Compounds considered to be important in the flavor of French-fried potato are 2,4-dimethyl-5-propyloxazole, 2-methyl-4-butyloxazole, and 2,4-dimethyl-5-butyloxazole, which have characteristic strong green, herbal, and vegetable-like aromas, and 2-pentyl-4-methyl-5-ethyloxazole, which is reported to have a strong buttery, sweet, and lactone-like flavor [86]. The last compound is thought to make an important contribution to the fried-food aspect of this flavor.

The exact mechanism of alkyloxazole formation is unclear; however, it is possible that they are formed by the condensation of hydroxylated amino acids or aminoketones with alkanals [73]. These reactions initially lead to the formation of oxazolines, which in turn can be oxidized to the corresponding oxazoles. These reactions can be considered part of the complex Maillard reactions taking place during the cooking of the potato. This reaction was discussed more fully under Baked Potato. However, nine of the compounds identified have long-chain (four carbon atoms or longer) alkyl substituents at the 2-position of the oxazole ring, and it has been suggested that their formation involves lipid or lipid decomposition products [86].

C. Jerusalem Artichoke

The tuber of the Jerusalem artichoke (Helianthus tuberosus L.) is normally eaten hot with the main meal but can also be eaten in stews and soups. The volatile components of the cooked vegetable have been isolated by combined steam distillation/solvent extraction at atmospheric pressure [87]. Twenty of the 52 components detected were identified by GC-MS, and quantitative data were obtained on all of the compounds detected. Sensory data were obtained, but no aromagrams were provided. The majority (20) of the compounds either identified or tentatively identified are aliphatic hydrocarbons, and surprisingly few aliphatic alcohol or carbonyl compounds, normally typical components of cooked vegetables, were detected. The major component is β-bisabolene (2.62 μg/kg), 51% of the total extract. β-Farnesene (0.13 μg/kg) was also identified. However, several compounds with aromas described as Jerusalem artichoke-like were not identified.

β-Bisabolene, because of its high concentration, could be expected to play a major role in the flavor of this vegetable even though it does not possess an aroma characteristic of the Jerusalem artichoke. Clearly the volatile components of this vegetable require detailed study.

D. Sweet Potato

The tuber of the sweet potato plant (Ipomoea batatas L. Lam.) is usually eaten hot as part of the main meal after it has been either boiled or baked. The volatile components of the sweet potato have been isolated by steam distillation at atmospheric pressure followed by solvent extraction [88], and from the baked vegetable by headspace concentration [89]. No quantitative data are available, and no aromagrams have been published. Identifications were obtained by the preparation of suitable derivatives [88] and by the examination of extracts by GC-MS techniques [89].

A total of 14 compounds were identified in the steam distillate from the boiled tubers, and these include four simple aliphatic aldehydes, two aliphatic

tannia (Xanthosoma sagittifolium), arrowroot (Maranta arundinacea), oca (Oxalis tuberosa), ulluco (Ullucus tuberosus), and ysano (Tropaeolum tuberosum). Several of these vegetables are important carbohydrate sources in underdeveloped countries, and as such they warrant investigation.

Figure 5 shows the chemical structures of some major constituents of tuber vegetables.

A. Potato

1. Introduction

The tuber of the potato (Solanum tuberosum L.) is eaten only after cooking and can be served either hot with the main meal or cold in salads. Either sliced or whole it can be boiled, baked, or fried, and it can also be processed by methods such as drying, freezing, or canning before reheating. The volatiles of the various cooked forms of this vegetable have been the subject of considerable study, and the significant differences in the composition of the volatiles obtained from the major cooking procedures requires that they be discussed separately under the headings of boiled, baked, potato chips, and French-fried.

2. Boiled Potato

A total of about 140 compounds have been identified as volatile components of raw, boiled, and dehydrated potato [3]. The volatiles were isolated from cooked and reconstituted dehydrated material by combined steam distillation/solvent extraction at both atmospheric pressure [63,64] and reduced pressure [63,65], and by headspace sampling [66,67]. Raw potatoes were also extracted using the vacuum steam distillation/solvent extraction technique [63,65]. The compounds were identified by GC-MS, and aromagrams of cooked, peeled, and unpeeled potatoes [64] (Figs. 6 and 7) and of reconstituted potato granules have been published [66]. In this

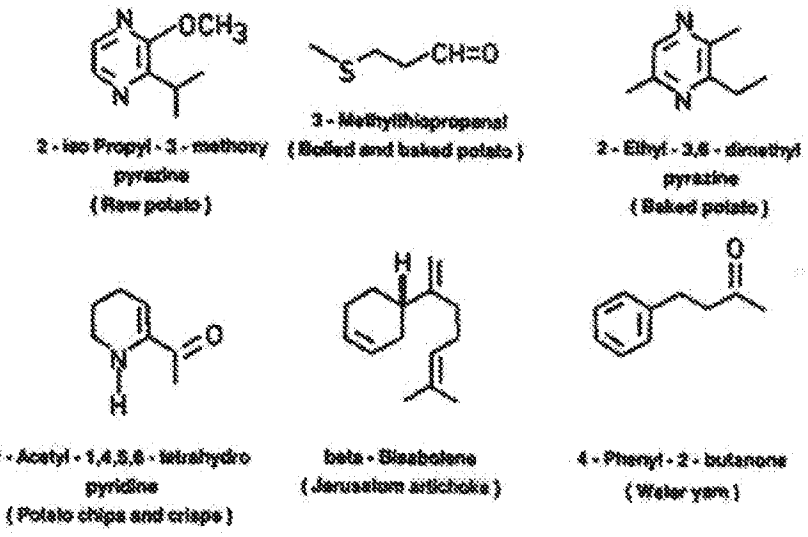

Figure 5 Some compounds important in the flavor of tuber vegetables.

E:N (essential amino acids to nonessential amino acids), E:P (essential amino acids to protein), and E:T (essential amino acids to total amino acids) ratios, chemical score, EAAI, and BV in rye, triticale, and wheat are presented in Table V. The results show that E:N and E:T ratios are highest in triticale, followed by wheat, Kalyan Sona, and rye. The results obtained for chemical score, EAAI, and BV in the cases of wheat and rye agree very closely with those of Sharbati Sonora and rye reported by Eggum (1970) and Duggal and Eggum (1977). As observed from Table V, PER is well correlated with chemical score in the case of rye and wheat, but this is not true in the case of triticale. Superiority in terms of PER, NPR, and growth of triticale over wheat in this study could not be due to essential amino acids, but it could be due to some other unknown factors, such as digestibility.

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Volatile Aroma Components of Cooked Artichoke

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The volatile oil of artichokes (*Cynara scolymus*), obtained by atmospheric steam distillation continuous extraction, was analyzed by the direct combination of capillary gas chromatography and mass spectrometry. A total of 32 compounds were characterized. The major components were β-selinene and caryophyllene. Odor threshold determinations indicated that the components most important to the aroma included oct-1-en-3-one, hex-1-en-3-one, decanal, non-*trans*-2-enal, phenylacetaldehyde, and eugenol.

California is the main growing area for artichokes (*Cynara scolymus*) in the United States. An improved knowledge of the aroma constituents of artichokes could give a better basis for breeding for improved flavor in the production of artichokes. There have been a number of studies on the nonvolatile constituents of artichokes particularly in regard to bitter off-flavor components such as the sequiterpene lactones (Samek et al., 1971; Schneider and Thiele, 1974). However, there does not seem to have been any previous reports on the volatile aroma constituents of artichoke. The present work was begun with the purpose of characterizing the major important volatile aroma constituents.

EXPERIMENTAL SECTION

Materials. Whole fresh California artichokes (*Cynara scolymus*) were obtained from local retail markets.

Authentic samples of organic compounds were obtained from reliable commercial sources or synthesized by established methods. They were purified by gas–liquid chromatography (GLC) separation before use.

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Isolation of Volatile Oil. Fresh artichokes (3 kg) were cut into quarters and placed in a 12-L round-bottom flask. They were covered with odor-free water (6 L) and a Likens-Nickerson steam distillation continuous extraction head attached to the top of the flask. Freshly distilled diethyl ether (150 mL) containing a trace of Ionox 330 antioxidant was placed in a flask attached to the solvent arm of the head. The extraction was carried out at atmospheric pressure for 3 h. After drying over anhydrous sodium sulfate, the ether was removed by distillation through low hold-up Vigreux distillation columns to give the artichoke volatile oil.

For separation into hydrocarbon and oxygenated fractions, the artichoke volatile oil (50 μL) was placed on a column (12 × 100 mm) of silica gel (Mallinckrodt SilicAR CC-7). The hydrocarbon fraction was eluted with pentane (200 mL). The oxygenated fraction was then eluted with freshly distilled diethyl ether (200 mL). Solvent from both fractions was removed by distillation using low hold-up distillation columns.

Capillary GLC–Mass Spectral Analysis. This was carried out in a similar way to that previously described by the authors (Buttery et al., 1975). In the present work, two major types of capillary columns were used: a 150 m long × 0.75 mm i.d. Pyrex glass capillary column coated

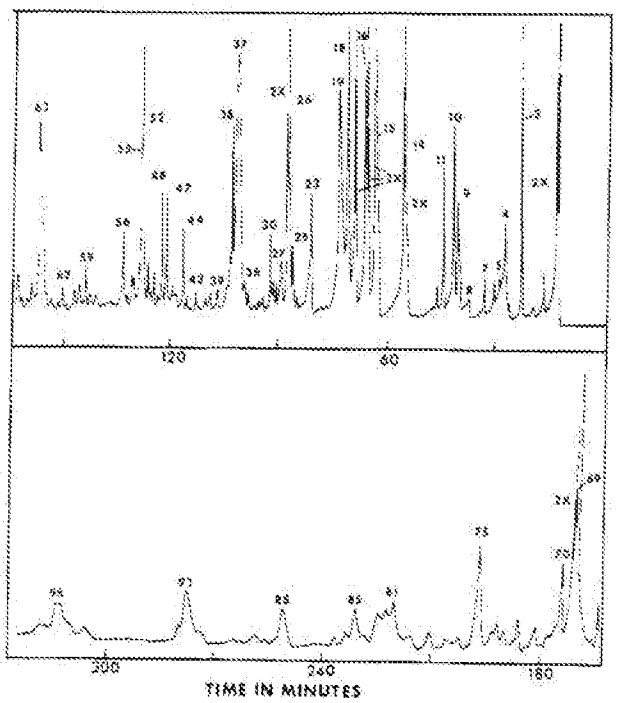

Figure 1. Capillary GLC analysis of the oxygenated fraction of the atmospheric steam volatile oil of artichokes. GLC column was a 150 m long × 0.75 mm i.d. Pyrex glass capillary coated with Tween 20 containing 5% Igepal CO-880. The column was held at 50 °C for 12 min after injection and then temperature programmed from 50–170 °C at 1 °C min.

with Tween 20 containing 5% Igepal CO-880 and a 300 m long × 0.75 mm i.d. stainless steel capillary coated with Silicone SF96(50) containing 5% Igepal CO-880. With these columns several different GLC–mass spectral runs were made using different GLC conditions. The main one was, however, that using the Pyrex glass capillary, holding at 50 °C for the first 12 min after injection and then temperature programming from 50–170 °C at 1 °C/min. A silicone rubber membrane molecular separator was used to couple the end of the capillary GLC column to the mass spectrometer (a modified Consolidated 21-620 cycloidal type). Electron ionization voltage was 70 V.

Packed Column GLC–Infrared Spectral Analysis. Samples were separated from both the hydrocarbon and oxygenated fractions using a 3 m long × 0.64 cm o.d. stainless steel column packed with 80–100 mesh Chromosorb G-DMCS coated with 2% silicone SF96(50). The column was temperature programmed nonlinearly from 50–170 °C. The infrared (IR) absorption spectra were measured as films between ultramicro salt plates or as solutions in CCl_4 in ultramicrocavity cells using a reflecting beam condensor with a Perkin-Elmer Model 237 instrument.

Odor Thresholds. These were measured in water solution (on the GLC purified compounds) as previously described (Guadagni et al., 1963) using Teflon bottles and tubing for the odor solutions.

RESULTS AND DISCUSSION

The raw artichoke has little aroma and flavor, and artichokes are usually eaten cooked. For this reason, it was felt that steam distillation continuous extraction at atmospheric pressure would be a satisfactory method of isolating the volatile oil. Using this method, a volatile oil amounting to 10 parts per million (ppm) of the artichoke was obtained. This oil was judged by four experienced odor judges to have an odor very similar to that of cooked artichokes. The whole volatile artichoke oil was first examined by the direct combination of capillary GLC and mass spectrometry (GLC–MS) and major components characterized. For a more detailed study, the whole oil was separated into a hydrocarbon fraction and an oxygenated fraction by selective liquid chromatography separation on a silica gel column. These fractions were found to be roughly 60% (hydrocarbon) and 40% (oxygenated) of the whole oil. Each fraction was then separately analyzed by GLC–MS and also by packed column GLC isolation of selected peaks for infrared spectral characterization.

Figure 1 shows a capillary GLC analysis of the oxygenated fraction. Components characterized in this and the hydrocarbon fraction are listed in Table I. Peak numbers corresponding to the peaks in Figure 1 are shown in parentheses immediately before the compounds name in Table I. Compounds not assigned a peak number are either hydrocarbons (hence not in oxygenated fraction) or

Table I. Identities of Constituents of the Steam Volatile Oil of Artichokes

Compound	Approx. relative percent in whole oil
Aliphatic alcohols[a,b]	
(9) 2-Methylbutanol MS, RT	0.9
(9) 3-Methylbutanol MS, RT	
(14) 3-Methylbut-2-en-1-ol MS, RT	2.2
(16) Hex-*cis*-3-enol MS, RT	3.5
(17) Hex-*trans*-2-enol MS, RT	1.3
(18) Hexanol MS, RT	2.7
Aliphatic aldehydes	
Pentanal MS, RT	0.2
(5) Hexanal MS, RT	0.5
Heptanal MS, RT	0.2
Octanal MS, RT	0.2
(19) Nonanal MS, RT	0.6
Decanal MS, RT	0.4
(10) Hex-*trans*-2-enal MS, RT	1.6
Non-*trans*-2-enal MS, RT	0.2
Aliphatic ketones	
Penian-2-one MS, RT	0.1
Butan-2,3-dione MS, RT	0.1
(4) Hex-1-en-3-one MS, RT	0.8
Oct-1-en-3-one, MS, RT	0.6
Aromatic and heterocyclic compounds	
Pyridine MS, IR, RT	0.2
2-Pentylfuran MS, RT	0.9
(18) Furfural MS, IR, RT	2
2-Acetylthiazole MS, RT	0.1
(23) Benzaldehyde MS, IR, RT	0.8
(37) Phenylacetaldehyde MS, RT	7
(52) Benzyl alcohol MS, RT	1
(69) Eugenol MS, IR, RT	2–5
Terpene alcohols	
(30) Linalool MS, RT	0.4
α-Terpineol MS, RT	0.2
(44) Linalool oxide C (5-hydroxy-2,6,6-trimethyl-2-vinyltetrahydropyran) MS, RT	0.5
Sesquiterpene hydrocarbons	
Caryophyllene MS, IR, RT	19
Humulene MS, RT	1
β-Selinene MS, IR, RT	40
Tentatively identified compounds	
(26) Methylheptanol	3.5

[a] Peak numbers corresponding to peaks in Figure 1 are shown in parentheses immediately before the compounds name. [b] MS, IR, RT = mass spectral, infrared absorption spectral, and GLC retention evidence, respectively. Evidence cited is consistent with that of an authentic sample.

Table II. Odor Thresholds and Calculations of Relative Odor Units of Some Artichoke Components

Component	Threshold (T_e) in parts of compound per 10^9 parts of water	Rel % of whole oil	Odor units, $U_o \times 10^{-6}$	% odor units
Whole artichoke oil	0.6	100	1690	100
Oct-1-en-3-one	0.005	0.6	1200	71
Hex-1-en-3-one	0.024	0.8	330	20
Non-*trans*-2-enal	0.08	0.2	25	1.5
Decanal	0.1	0.4	40	2
Octanal	0.7	0.2	3	0.2
Nonanal	1	0.6	6	0.4
Heptanal	3	0.2	0.7	0.04
Phenylacetaldehyde	4	7	20	1.1
Hexanal	4.5	0.5	1	0.06
Eugenol	6	5	8	0.5
Linalool	6	0.4	0.7	0.04
2-Pentylfuran	6	0.9	2	0.1
Pentanal	12	0.2	0.2	0.01
Hex-*trans*-2-enal	17	1.6	0.9	0.05
Caryophyllene	64	19	3	0.2
Hex-*cis*-3-enol	70	3.5	0.5	0.03
3-Methylbutanol	250	0.9	0.04	0.002
Benzaldehyde	350	0.8	0.02	0.001
α-Terpineol	350	0.2	0.006	0.0004
Hexanol	500	2.7	0.05	0.003
Furfural	3000	2	0.007	0.0004

are covered by other peaks in this particular GLC analysis. Different GLC conditions favored some compounds over others. Some idea of the approximate relative percentages of the components in the whole oil (calculated from peak areas) is also listed in Table I.

The major components of the volatile oil are the sesquiterpene hydrocarbons β-selinene and caryophyllene forming 40 and 19%, respectively, of the whole oil. These compounds do not seem to be closely related to the nonvolatile sesquiterpene lactones previously characterized in artichokes (Samek et al., 1971). More than ten volatile oxygenated sesquiterpenoids (mostly alcohols), occurring in relatively small concentration, were detected but none could be characterized from their mass spectra. There are a number of aliphatic alcohols occurring in reasonable concentration such as hex-*cis*-3-enol (3.5%) and hexanol (2.7%) which are common in many vegetables and fruits. An unusual aliphatic alcohol occurring in reasonable concentration (2.5%) is peak 26. This compound has a mass spectrum very similar to that of 6-methylheptanol, but its GLC retention is slightly shorter. The mass spectrum is different from that of 2-methylheptanol and the branch may possibly be in the 3 or 4 or 5 position. No authentic samples of these isomers were available, however.

As far as the authors can determine, hex-1-en-3-one has not been found in foods before. Oct-1-en-3-one had been found previously in dairy products (Forss et al., 1962), mushrooms (Cronin and Ward, 1971), and potatoes (Buttery et al., 1970) but is not widely occurring although the corresponding alcohol oct-1-en-3-ol has been found in a large number of foods. Both of these ketones are potent odorants.

Most of the other components characterized such as the aliphatic aldehydes, terpene alcohols, and aromatic and heterocyclic compounds commonly occur in vegetables.

The mass spectra of most of the components are well enough known. The mass spectrum of hex-1-en-3-one (two most intense ions every 14 mass units above m/e 34, intensities in parentheses, molecular ion in boldface type) showed 41 (45), 43 (59); 55 (100), 56 (13); 70 (42), 71 (12), 83 (12), 84 (0.5); 97 (6), **98** (10).

Importance of Components to Aroma. During the capillary GLC analysis of the whole artichoke volatile oil, the odor of the effluent was informally evaluated to determine the odor quality of the components. The only individual GLC peak that was felt to have a characteristic artichoke aroma was that corresponding to hex-1-en-3-one. The peak corresponding to oct-1-en-3-one had a mushroom-like odor.

Odor thresholds (T_e) in water of most components had been determined previously by the authors during studies on other foods (e.g., Buttery et al., 1970; Guadagni et al., 1966). These are listed in Table II together with thresholds determined for the present study. The percentage of the component in the oil is also shown, and these data were used to calculate odor units (U_o) for each compound (cf. Guadagni et al., 1966). If we consider the concentration of each component in the oil in parts per billion (ppb) expressed as F_c, then odor units have been defined by Guadagni et al. (1966) as $U_o = F_c/T_e$. The percent odor units is also shown. This gives some idea of the relative importance of the components to the total odor. The most important odorants seem to include oct-1-en-3-one, hex-1-en-3-one, decanal, non-*trans*-2-enal, phenylacetaldehyde, and eugenol. Mixtures of dilute solutions of some of these components in informal evaluations gave aromas reminiscent of cooked artichokes.

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